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ONSET OF COCCIDIOIDOMYCOSIS IN MOUSE LUNG AFTER INTRAVENOUS INJECTION

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UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

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and
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In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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ABSTRACT

Intravenous injection of mice with a massive dose of Coccidioides immitis fungal elements caused a moderate inflammatory response after 6 hours. It was composed of small focal collections of lymphocytes and neutrophils surrounding the rounded fungal elements in the mouse lungs. No further change was noted at 24 hours. Spherules with endospores varying in diameter from 15 to 40 microns were seen at 48 and 54 hours. Neutrophils persisted throughout this time and increased only minimally; the lymphocytic response was more marked at these later observations.

I. INTRODUCTION

Studies of the histologic responses of the host to infection by Coccidioides immitis have not been described for the very early stages of the disease. Forbus and Bestebreurtje[1] studied tissues obtained from patients at autopsy; Tager and Liebow[2] used mice that were sacrificed at various times after infection. The latter used intranasal and intraperitoneal methods of infection, but could not trace the development of the tissue response between 9 hours and 4 days. Tarbet et al.[3] used the intraperitoneal route of injection and found the first evidence of response at 20 hours after injection. In studies both of tissues from patients with coccidioidomycosis and of mice experimentally infected, a regular pattern of response was observed: Spherules attracted mononuclear cells; these accumulated and almost completely replaced the polymorphonuclear leukocytes that had dominated the earliest stages in the development of the spherule.

The present investigation was designed to observe in the mouse lung, the early stages of tissue response to large numbers of viable C. immitis arthrospores and hyphal fragments injected intravenously. The route and large dose used would allow very early accurate observations of the tissue reactions.

II. MATERIALS AND METHODS

Mature, male, white, Swiss mice, Webster strain, born and raised at the Fort Detrick animal farm and weighing 25 to 30 grams each were used for this investigation. The inoculum, C. immitis, strain Silveira, grown on Sabouraud agar slants for 4 months at 34 C, was removed from the slants by scraping with a stiff wire after the addition of a 0.01% aqueous solution of triethanolamine oleate. The fungal elements were broken up by shaking with glass beads. Ten animals received an injection of 0.25 ml of the fungal suspension and the remaining seven received 0.1 ml, shown by plate counts to contain 11,600,000 and 2,900,000 viable particles respectively. Two animals receiving the higher dose died immediately after injection.

The animals that survived the challenge inoculation were sacrificed by an overdose of Nembutal, three each at 6, 24, 30, 48, and 54 hours after challenge. Animals receiving both dose levels were sacrificed at each time interval, except the 30-hour group, which consisted of three animals receiving the lower dose. One noninfected control animal was sacrificed at the 54-hour period. The lungs of all animals were fixed in 10% buffered formalin; tissue sections were stained by the Giemsa or Gomori technique.

A second series of 21 animals were similarily injected, but with dead arthrospores. One million dead arthrospores, as determined by direct microscopic count, in 0.5 ml of TEO solution, were administered to each animal intravenously. Three animals each were sacrificed at 0, 19, 25, 43, 50, 68, and 73 hours. Animals were sacrificed and tissue preserved as previously described.

III. RESULTS

In the two animals that died immediately after challenge, the pulmonary vessels were diluted and contained tangled masses of branching septate hyphae, some of which appeared to be swelling and assuming a spherical form (Figure 1). It is assumed that this swelling occurred before injection and was not a result of interaction with the mouse tissue. These fungal elements, although visible with the Giemsa stain, were clearer and more prominent with the Gomori stain. No cellular reaction was elicited at this time. One mouse had pre-existing chronic murine pneumonia (CMP) in one lung. A few fungal elements were also seen in the large vessels of the heart and liver. The spleen and lymph nodes exhibited hyperplasia of the lymphocytic elements.

Six hours after challenge, the pulmonary vasculature again contained tangled masses of fungal elements. However, a moderate inflammatory response, characterized by small focal collections of lymphocytes with some neutrophils, was noted for the first time. In most instances the inflammatory cells surrounded the rounding arthrospores and hyphal elements.

At 24 hours the only animal receiving the larger challenge dose exhibited CMP in one lung; however, the opposite lung was similar to that seen in the 6-hour sacrifice except that there was an increase in the size and number of cellular collections around the fungus cells. Interestingly enough, the two mice receiving the smaller challenge exhibited a more marked cellular response. There was no increase in the number of neutrophils scattered among the lymphocytes; fungal elements increased in number and appeared rounded (Figure 2). They measured 6 to 8 microns in diameter.

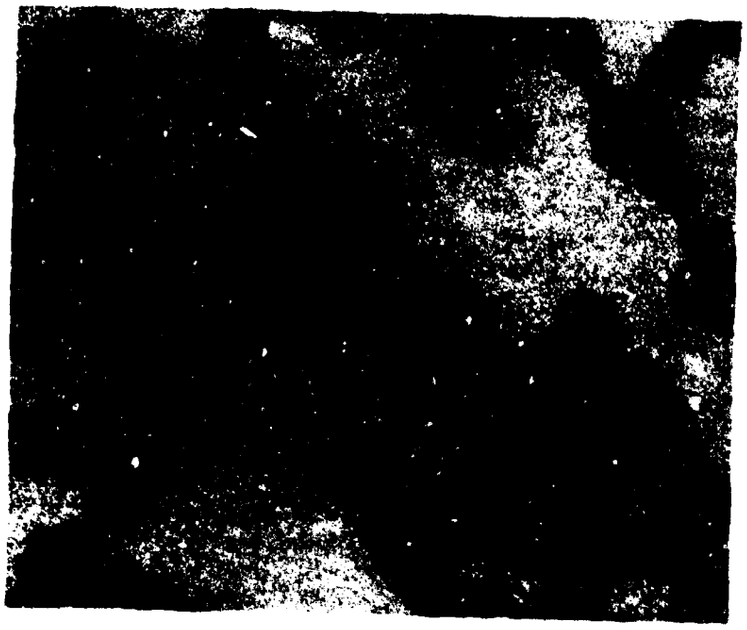

Figure 1. Tangled Mass of Fungal Elements in the Pulmonary Vasculature Immediately after Injection. Gomori Stain, 350X.

Figure 2. Typical Rounded Form Seen in the Lungs at 24 Hours. Gomori Stain, 350X.

The three mice sacrificed at 30 hours showed essentially the same histopathological changes as the 24-hour animals. However, at 48 hours, regardless of dose, definite spherules were seen for the first time (Figure 3) and they increased in size and number by 54 hours (Figure 4). The spherules varied in size from 15 to 42 microns and were in various stages of development. Endospores were observed at 48 and 54 hours. Neutrophils persisted for the duration of the experiment and increased only slightly in number in the later stages. In general, the lymphocytic response was more marked at this time. The uninoculated control mouse sacrificed at 54 hours was not remarkable.

In the second portion of the experiment, in which mice were injected intravenously with dead arthrospores, the earliest histopathologic changes were seen in the lungs at 19 hours. These consisted of small focal collections of lymphocytes containing a few neutrophils. This pattern persisted throughout the experiment and did not change appreciably with time. At no time were fungal elements demonstrated.

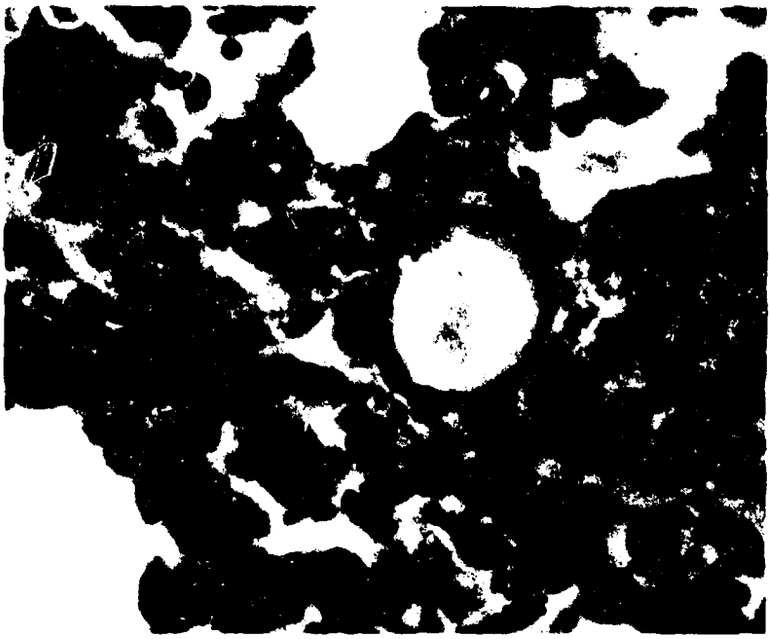

Figure 3. Spherule Surrounded by Lumphocytes and an Occasional Neutrophil at 48 Hours. Giemsa Stain, 350X

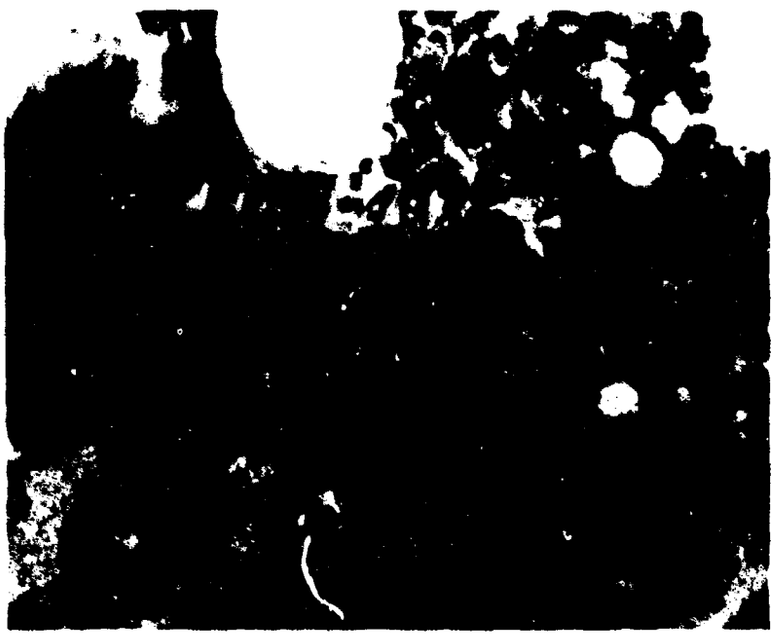

Figure 4. Spherules in Various Stages of Development in Lungs of Mouse Sacrificed at 54 Hours. Note that the largest spherule is slightly out of focus but apparently ready to release endospores. Neutrophils can be readily identified. Giemsa Stain, 250X.

IV. DISCUSSION

Tarbet et al.[3] in his comprehensive report stated that young spherules elicited a neutr phili c cellular response, whereas the mature forms attracted epithelioid cell . Release of endospores again elicited neutrophils and the cycle was repeated. However, in contrast to the findings of Tarbet et al. the response in this investigation was primarily lymphocytic. As early as 6 hours, the principal cellular collections surrounding the invading fungus cells were lymphocytic, with only a few neutrophils. As the spherules matured there was a marked increase in the size of the inflammatory foci but the number of neutrophils increased only slightly. At no time were epithelioid or giant cells seen. Because no endospores were released, there was no opportunity to study the later aspects of the cycle described by Tarbet et al.

Spherule development following intravenous injection was similar to that reported by other workers.[3,4] Rounded forms were seen at 24 hours that could have been young spherules (Figure 2). Definite spherules with endospore formation were observed at 48 and 54 hours.

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